The Effect of *RANTES* Chemokine Genetic Variants on Early HIV-1 Plasma RNA Among African American Injection Drug Users

Priya Duggal, PhD, MPH, Cheryl A. Winkler, PhD,† Ping An, MD,† Xiao-Fang Yu, ScD, MD,‡ Homayoon Farzadegan, PhD,*‡ Stephen J. O'Brien, PhD,§ Terri H. Beaty, PhD,* and David Vlahov, PhD*‖*

Summary: HIV-1 plasma RNA is a prognostic indicator of HIV-1, and increased levels of HIV-1 plasma RNA are associated with rapid progression to AIDS. Because chemokines and chemokine receptors are involved in the binding and entry of HIV-1, possible effects of host genetics on viral RNA levels should be visible in early infection. HIV-1 plasma RNA was measured within 2 years of seroconversion in 198 seroincident injection drug users followed in the AIDS Link to Intravenous Experience cohort. Genetic variants were identified in the chemokine receptors (*CCR2*, *CCR5*, and *CCR5* promoter) and the chemokine *RANTES* using TaqMan and restriction fragment length polymorphism assays. Linear regression of *RANTES* haplotypes on early HIV-1 plasma RNA identified individuals homozygous for the *RANTES* R1 haplotype as having a lower viral load by almost one-half $\log_{10}$ unit compared with those bearing non-*RANTES* R1 haplotypes (−0.43, 95% confidence interval: −0.74, −0.12). Genetic variants in *RANTES* may downregulate RANTES gene expression and increase early HIV-1 plasma RNA. Because RANTES is a critical chemokine and competitively inhibits HIV-1 by binding to its receptor CCR5, treatment to enhance RANTES expression may assist in delaying the progression of AIDS by decreasing the initial viral load.

Key Words: chemokines, RANTES, HIV-1 plasma RNA, HIV

(*J Acquir Immune Defic Syndr* 2005;38:584–589)

Received for publication February 11, 2004; accepted May 24, 2004.

From the *Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; †Basic Research Program, Science Applications International Corporation (SAIC) Frederick, National Cancer Institute, Frederick, MD; ‡Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; §Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD; and ‖Center for Urban Epidemiologic Studies, New York Academy of Medicine, New York, NY.

Funded in part with Federal funds from the National Cancer Institute, National Institutes of Health, under contract N01-CO-12400 and by the National Institute of Drug Abuse, grant DA 04334.

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Reprints: David Vlahov, Center for Urban Epidemiologic Studies, The New York Academy of Medicine, 1216 Fifth Avenue, New York, NY 10029–5293 (e-mail: dvlahov@nyam.org).

Chemokines and their receptors play a critical role in HIV-1 binding and entry. Chemokine receptors function as viral coreceptors with CD4 to permit binding and entry of HIV-1 into macrophage and T cells. The principal chemokine receptors in HIV-1 transmission and progression are CCR5 for the R5 (macrophage tropic) and CXCR4 for the X4 (T-cell tropic) viruses. Other chemokine receptors, including CCR2, may act as secondary coreceptors.[1]

The chemokine RANTES is one of 3 natural ligands for CCR5. Chemokines interfere with the spread of HIV-1 by 2 mechanisms: by competitively binding to their respective receptors, they block binding of HIV envelope glycoprotein, gp120, and by inducing internalization of the bound receptor, thereby reducing coreceptor availability.[2] Studies have shown a decline in RANTES levels with HIV disease progression[3] and increased production of β-chemokines, including RANTES, among HIV-exposed uninfected individuals.[4–7]

Genetic epidemiologic cohort studies have shown that polymorphisms in the genes encoding these chemokines and chemokine receptors are associated with altered rates of disease progression after HIV-1 infection. Genetic variants of *CCR2*, *CCR5*, and its ligand, *RANTES*, have been associated with both delayed disease progression (*CCR5*-Δ32, *CCR2*-64I, and *RANTES* −403A) and accelerated disease progression (*RANTES* In1.C and *CCR5*-P1).[8–16]

Although this epidemiologic evidence has aided our understanding of the importance of chemokines and chemokine receptors in HIV-1 disease progression, the effect of *RANTES* variant alleles on HIV-1 RNA levels has not been demonstrated. Clinical studies of the *CCR5* genotypes have shown decreased early HIV-1 plasma RNA for individuals heterozygous for *CCR5*-Δ32.[17–19] The underlying mechanism of the 2- to 4-year delay in progression to AIDS-defining conditions afforded by *CCR5*-Δ32 in heterozygotes may result from a decrease in available CCR5.

The *CCR5*-P1 promoter haplotype has been associated with accelerated progression to AIDS in both European and African Americans.[8,20] Unlike *CCR5*Δ32, which is rare or absent in non-Europeans, *CCR5-P1* is quite frequent in all tested populations (frequency between 10% and 35%).[20,21] In African Americans, the effect of *CCR5-P1* is codominant, suggesting that the *CCR5* promoter haplotypes differentially regulate transcription levels of CCR5. A single nucleotide

polymorphism (SNP) within the *CCR5-P1* haplotype has been associated with increased transcription of the *CCR5* gene.[22]

SNPs in the *RANTES* regulatory regions have been shown to modify RANTES transcription in reporter assay systems.[9,11] Two variant alleles, −28G and −403A, in the promoter have been shown to upregulate RANTES transcription in reporter assays and to modify rates of HIV-1 progression in some studies. A variant allele in the 3′-untranslated region 3′222C) has been suggested as an HIV/AIDS genetic modifier.[9] Another variant allele, In1.1C, occurs in an enhancing regulatory element in the first intron of the *RANTES* gene and was found to downregulate transcription in reporter assays.[9] Because −28G, −403A, In1.1C, and 3′222C are in strong positive disequilibrium and both In1.1C and −28G always occur on a −403A-bearing haplotype, it is difficult to separate the independent effects of the 4 variant alleles, although functional assays suggest that the In1.1T/C site is likely causal. All these alleles are in strong positive linkage disequilibrium and form 4 common haplotypes, with the most frequent being the *R1* haplotype with the common allele at positions −403, −28, In1.1, and 3′222.

A key prognostic indicator of HIV-1 disease progression is the initial level of HIV-1 plasma RNA. Elevated levels of HIV-1 plasma RNA are associated with fast progression to AIDS in this cohort and others.[23,24]

In this study, we sought to determine whether genetic variants in the *CCR5*-promoter and in the *RANTES* gene encoding the CCR5 ligand altered the initial HIV-1 plasma RNA in a cohort of African American seroincident injection drug users (IDUs). Here, we test the hypothesis that the *CCR5*-promoter *P1* haplotype and *RANTES* haplotypes previously shown to modify HIV-1 progression to AIDS are associated with HIV-1 plasma RNA levels in a group of seroincident IDUs.

METHODS

Study Population

As previously described, from February 1988 to March 1989, IDUs from the Baltimore community were recruited and screened for HIV antibodies and invited to join a prospective cohort study on the natural history of HIV, the AIDS Link to the Intravenous Experience (ALIVE).[25] Individuals were considered eligible if they were at least 18 years old, had engaged in injection drug use within the previous 10 years, and had not experienced an AIDS-defining illness. The Institutional Review Board of the Johns Hopkins Bloomberg School of Public Health approved all study and consent procedures.

Data Collection

The early viral load was defined as the first viral load measurement within 2 years after the estimated date of seroconversion. There were 250 participants in the study who seroconverted during the study period and had initial plasma HIV-1 RNA. Of these, 11 were ineligible, because the initial viral load measurement was more than 2 years after seroconversion. Additionally, 15 individuals were not African American and were excluded because of the potential for confounding as a result of population heterogeneity. Of the 224 individuals with HIV-1 seroconversion who met the criteria for inclusion, 198 were genotyped at the Laboratory of Genomic Diversity, Frederick, Maryland, for each of the following genotypes: *CCR5*-Δ32; *CCR2*-64I; *CCR5* P1; and *RANTES* −28G, −403A, In1.1C, and 3′222. Haplotypes were inferred using the maximum likelihood method. For comparability, we describe the CCR5 variants using both the Laboratory of Genomic Diversity and Tri-Service HIV-1 Natural History Study (TSS) nomenclature.[20,26]

Laboratory Techniques

Antibodies to HIV-1 were detected using an enzyme-linked immunosorbent assay (ELISA) (Genetic Systems, Seattle, WA) with confirmation of positive ELISA tests by Western blot analysis (DuPont, Wilmington, DE). HIV-1 viral load was assessed using the reverse transcriptase polymerase chain reaction (RT-PCR) assay (Roche Molecular Systems, Branch-burg, NJ). The minimum detectable level of HIV-1 RNA was 400 copies/mL; levels that were read as undetectable were coded for analysis as 200 copies/mL. PCR restriction fragment length polymorphism (RFLP) and 5′ nuclease PCR (TaqMan) assays were used for genotyping. Genotyping was performed as previously described.[8,9,14,16,27]

Statistical Analysis

The seroconversion date was estimated as the midpoint between the last HIV-negative visit date and the first HIV-positive visit date. HIV-1 RNA measurements were transformed to $\log_{10}$, and early HIV-1 plasma RNA was used as the outcome. The *RANTES* haplotypes (Table 1) were determined using an expectation maximization (EM) algorithm that first recursively computes the expectation of the haplotype and then maximizes the expected likelihood until a convergence criterion is met to determine population-based haplotypes, as previously described.[9] The *CCR2-CCR5P1-CCR5* haplotype was determined by counting and resulted in 4 haplotypes (Table 2). Individuals homozygous for *CCR2*-64V, *CCR5*-P1, and *CCR5*+ are also referred to as E/E (TSS nomenclature). The Wilcoxon rank sum test was used for comparisons of continuous variables. The χ^2 test was used for comparisons of categoric variables. General linear model regression statistics were used to assess the association of individual genotypes or haplotypes with plasma HIV-1 RNA levels. To assess confounding, gender, age at seroconversion, seroconversion date, time from seroconversion to early viral load measurement, and other genotypes (possible genotypes included *CCR5*Δ32, *CCR2*V64I, *CCR5*-P1, *RANTES* −403, *RANTES* −28, *RANTES* In1.1, and *RANTES* 3′222) were included as

TABLE 1. The Haplotype Structures of *RANTES* for African American Participants of the ALIVE Cohort

Haplotype	−403	−28	In1.1	3′222
R1	G	C	T	T
R2	A	C	T	T
R3	A	C	C	T
R4	A	C	C	C

G, A, C, and T refer to base pairs.

TABLE 2. Haplotypes for *CCR2, CCR5 Promoter, CCR5* Among ALIVE African American IDUs

Haplotype	*CCR2*	*CCR5* Promoter	*CCR5*
+/P1/+*	V64	*P1*	+
+/PX/+	V64	*PX*	+
+/P1/Δ32	V64	*P1*	Δ32
64I/P1/+	64I	*P1*	+

P1 indicates *CCR5* promoter P1 haplotype; PX, *CCR5* promoter P2 to P4 haplotypes; 64V/*CCR2* valine-to-isoleucine polymorphism at codon 64; Δ32, *CCR5* 32 base-pair deletion.

*Also referred to as haplotype E.

covariates in a multivariate model. All statistical analyses were done using STATA, version 6.0 (College Station, TX).

RESULTS

Of the 198 individuals with both early plasma HIV-1 RNA and genotype information, 151 were men (76%) and 47 were women (24%), which is consistent with the gender distribution among all seroincident cases in this cohort. The median early HIV-1 RNA for men (4.62, interquartile range [IQR]: 4.04–5.0) and women (4.23, IQR: 3.86–4.64) differed by 0.40 $\log_{10}$ units, which is consistent with previous studies showing a lower initial viral load for women compared with men.[28–30] Individuals with higher early HIV-1 plasma RNA levels progressed to AIDS faster than individuals with lower early HIV-1 plasma RNA measurements (data not shown), as seen in this and other cohorts previously.[23,24]

There were 4 haplotypes of *CCR2*-V64I: *CCR5* P1/other: *CCR5*+/Δ32 alleles (see Table 2). Additionally, there were 4 previously established *RANTES* haplotypes from the alleles (−403, −28, In1.1, and 3′222) that were also included (see Table 1).[9] Simple linear regression of initial HIV-1 plasma RNA on individual genotypes for each marker identified *RANTES* 403A and *RANTES* In1.1C as having an effect on HIV-1 plasma RNA (Table 3). Because these alleles are in strong or complete linkage disequilibrium with one another, a haplotype analysis was necessary to determine their combined effect. The −28 site, polymorphic in both Asians and Europeans, is not polymorphic in African Americans. Our cohort of African Americans did not exhibit any variance at the *RANTES* −28C/G promoter allele (100% were −28C/C), so this SNP was not included in the haplotype analysis.

Univariate linear regression of early HIV-1 plasma RNA and *RANTES* haplotypes identified the homozygous *RANTES* R1 haplotype (carrying the most common allele at the 4 sites) as having a lower viral load by almost one-half $\log_{10}$ unit compared with that with any other *RANTES* haplotype (−0.43, 95% confidence interval [CI]: −0.74, −0.12; Table 4). A separate analysis by gender showed similar trends in men (−0.48, 95% CI: −0.82, −0.13) and women (−0.21, 95% CI: −0.88, 0.46), although statistical power was considerably less among women because of the small number (n = 47). Additionally, after adjusting for potential confounders, including the *CCR5*Δ32, *CCR2* V64I, and *CCR5* P1 promoter haplotypes, gender, age, seroconversion date, and the time from seroconversion to viral load measurement, the strength of the association was even stronger for the *RANTES* R1 haplotype (−0.49, 95% CI: −0.80, −0.17; Table 5). The R1 homozygous individuals have lower HIV-1 plasma RNA levels than individuals carrying at least 1 R2, R3, or R4 haplotype.

The *CCR5* promoter analysis did not identify any single haplotype/diplotype with significant influence on initial HIV-1 plasma RNA. Additionally, we analyzed the *CCR2 64I* allele, which has been associated with modest influences on HIV-1 disease progression and is located in close chromosomal proximity to *CCR5*. The *CCR2* variant did not alter the initial

TABLE 3. Univariate Linear Regression of Initial Log_{10} HIV-1 Plasma RNA by Individual Chemokine and Chemokine Receptor Genotypes

Gene	Genotype	n	Difference in Log_{10} HIV-1 Plasma RNA	95% CI	*P*
CCR5	+/+	192	—	—	—
	+/Δ32	7	−0.50	−1.10, 0.09	0.10
CCR2	+/+	149	—	—	—
	V/I and I/I	50	0.16	−0.01, 0.43	0.22
RANTES	403 G/G	67	—	—	—
	403 G/A	91	0.28	0.03, 0.53	0.03
	403 A/A	40	0.43	0.12, 0.74	0.007
	In1.1 T/T	120	—	—	—
	In1.1 T/C	67	0.28	0.04, 0.51	0.02
	In1.1 C/C	11	0.39	−0.09, 0.88	0.11
	3′222 T/T	168	—	—	—
	3′222 T/C	29	0.14	−0.17, 0.46	0.37
	3′222 C/C	1	0.81	−0.75, 2.38	0.31

+ indicates wild-type allele.

TABLE 4. Univariate Linear Regression of Early Log_{10} HIV-1 Plasma RNA by *RANTES* and *CCR5* Promoter Haplotypes

Haplotype	n	Mean HIV-1 Plasma RNA	Difference in Log_{10} HIV-1 Plasma RNA	95% CI	*P*
All other *RANTES* haplotypes	40	4.63	—	—	—
Heterozygous R1	91	4.47	−0.16	−0.45, 0.13	0.28
Homozygous R1	67	4.20	−0.43	−0.74, −0.12	0.007
All other *RANTES* haplotypes	123	4.38	—	—	—
Heterozygous R2	68	4.42	0.04	−0.19, 0.28	0.71
Homozygous R2	7	4.72	0.34	−0.27, 0.95	0.27
All other *RANTES* haplotypes	145	4.34	—	—	—
Heterozygous R3	48	4.52	0.18	−0.07, 0.44	0.16
Homozygous R3	5	5.05	0.71	0.0003, 1.41	0.05
All other *RANTES* haplotypes	168	4.38	—	—	—
Heterozygous R4	29	4.52	0.14	−0.17, 0.46	0.37
Homozygous R4	1	5.20	0.82	−0.75, 2.39	0.31
All other *CCR5* promoter haplotypes	83	4.40	—	—	—
Heterozygous CCR5 P1	82	4.49	0.09	−0.15, 0.344	0.45
Homozygous *CCR5* P1	33	4.28	−0.12	−0.44, 0.20	0.47

R1, R2, R3, R4, and P1 refer to haplotypes for *RANTES* and *CCR5* promoter region, respectively.

HIV-1 plasma RNA in our cohort, which may reflect a diminished role of this genetic variant on HIV-1 plasma RNA.

DISCUSSION

In this cross-sectional study, we analyzed early HIV-1 plasma RNA according to known modifying haplotypes in the chemokine coreceptor *CCR5* promoter, *CCR2*, and *RANTES* genes. We identified 0.49-log_{10} lower initial HIV-1 plasma RNA for those individuals homozygous for the *RANTES* R1 haplotype carrying the most frequent allele at the 4 polymorphic sites. Because RANTES is a critical ligand for CCR5, it was important to adjust for the effects of the *CCR5*-Δ32 mutation and its promoter haplotypes, because both the *CCR5*-Δ32 deletion and the *CCR5* promoter haplotypes have been shown to modify CCR5 surface expression and thereby interfere with HIV-1 replication kinetics. In univariate and multivariate analysis, the R1 haplotype maintained a lower initial viral load. Because the homozygous haplotype frequencies of R2 to R4 were quite small, we could not independently determine which allele might be responsible for the increased initial HIV-1 plasma RNA.

Earlier studies have shown increased rates of *RANTES* transcription with the −28G allele in a Japanese population.[11] In a study of at-risk HIV-seronegative individuals, those with the *RANTES* haplotype −403A, −28C had increased infection compared with those with the *RANTES* haplotype −403G, −28C. Additionally, seroconverters had a significantly slower progression to AIDS (under the 1993 criteria) for those carrying the *RANTES* −403A allele, suggesting that the *RANTES* −403A allele is a risk factor for acquiring HIV but may be protective in progression.[12] A global survey of *RANTES* −28C and −403A identified both as risk factors for HIV infection and accelerated progression to AIDS among European

TABLE 5. Multivariate Linear Regression of HIV-1 Plasma RNA by *RANTES* Haplotypes

Genotypes	n	Difference in Log_{10} HIV-1 Plasma RNA	95% CI	*P*
All other *RANTES* haplotypes	40	—	—	—
Heterozygous R1	91	−0.14	−0.43, 0.15	0.34
Homozygous R1	67	−0.49	−0.80, −0.17	0.003
All other *RANTES* haplotypes	123	—	—	—
Heterozygous R2	68	0.06	−0.18, 0.30	0.64
Homozygous R2	7	0.28	−0.33, 0.89	0.37
All other *RANTES* haplotypes	145	—	—	—
Heterozygous R3	48	0.20	−0.06, 0.47	0.13
Homozygous R3	5	0.74	0.03, 1.46	0.04
All other *RANTES* haplotypes	168	—	—	—
Heterozygous R4	29	0.19	−0.13, 0.50	0.24
Homozygous R4	1	0.72	−0.84, 2.28	0.36

R1, R2, R3, R4, and P1 refer to haplotypes for *RANTES*. Adjusted for *CCR2, CCR5,* and *CCR5* P1 haplotypes; gender; age; seroconversion date; and time from seroconversion to initial HIV-1 viral load measurement.

Americans homozygous for the *RANTES* promoter alleles −403A, −28C, but this association was not found in African Americans.[31] A recent study by An and colleagues[9] extended the haplotype region to include the *RANTES* SNP In1.C, which always occurs with the *RANTES* SNP −403A. In a survival analysis of 962 individuals (including ALIVE participants), the effect of the In1.1C allele and the composite *RANTES* R3 haplotype was associated with an accelerated progression to AIDS (relative hazard [RH] = 1.7; $P = 0.05$) and AIDS-related death (RH = 2.3; $P = 0.02$) among African Americans.[9] Further functional analysis determined that the In1.1C allele results in decreased rates of *RANTES* transcription and that relative to the In1.1T allele, the In1.1C allele downregulates *RANTES* transcription by approximately 4-fold.[9] We have recently shown that the In1.1T/C site occurs within a regulatory element that enhances transcription of *RANTES* and that this enhancement is abrogated by the In1.1C allele.[9] It is likely that the association of the *RANTES* R1 haplotype with lower HIV-1 RNA is a result of more abundant RANTES for CCR5 binding. Our finding validates the previously described association of accelerated disease progression to AIDS among those with genetic variants in *RANTES* by showing a correlation with increased initial HIV-1 plasma RNA.[9,11,12] In addition, these findings suggest that a potential mechanism for increased or decreased HIV disease progression is the level of RANTES expression controlled by these *RANTES* haplotypes. We did not determine if primary cells from individuals with different *RANTES* haplotypes produced different amounts of RANTES with antigenic stimulation, however. We would expect that R1 *RANTES* homozygotes would secrete more RANTES than cells from non-R1 *RANTES* individuals. This warrants further research to determine if the genetic differences in *RANTES* haplotypes can translate into significant differences in protein secretion over time.

Our analysis of the *CCR5* promoter P1 haplotype and its diplotypes (both haplotypes at a gene or locus) did not show a statistically significant difference on initial HIV-1 plasma RNA, unlike previous analyses. A viral load analysis of 341 white seroincident participants in the Multicenter AIDS cohort study (MACS) revealed a higher mean plasma HIV-1 RNA among E/E carriers (64V/*P1*/+) in the initial 42 months after seroconversion.[32] Additionally, African-American seroconverters with the E/E genotype had elevated HIV-1 RNA levels compared with other genotypes. In an additional study of predominantly African American women in the Reaching for Excellence in Adolescent Care and Health (REACH) study, 207 women tested seroprevalent and the E/E genotype was also identified as being associated with a higher plasma HIV-1 RNA level.[33]

We could not validate any influence of the E/E diplotype on initial HIV-1 plasma RNA or any effect of the *CCR5* P1 promoter allele. The accelerated disease progression noted in numerous cohort studies may be influenced by another variant in linkage disequilibrium with the *CCR5* P1 to P4 haplotypes. Although we detected an association with *RANTES*, we may have lacked adequate statistical power to identify a weaker association with *CCR5* P1. It is also plausible that the increased rates of progression to HIV-1 infection may not be driven by an increase in HIV-1 plasma RNA but through another mechanism altogether.

One limitation of this study is an incomplete understanding of the effects of genetic variants when there are differences in viral tropism. Individuals may be infected with R5 or X4 tropic virus, which can alter the utilization and significance of the RANTES chemokine. Within the ALIVE cohort, 46 seroconverters have been sequenced and/or characterized for viral tropism. Only 1 CXCR4 virus (2%) and 3 dual-tropic (CCR5 and CXCR4) viruses were detected. The remaining 42 seroconverters (91%) only had CCR5 virus detected. Even though not all the seroconverters in the ALIVE cohort were sequenced, these results suggest that this cohort of IDUs does exhibit a bias toward R5 transmission and would most likely use the *RANTES* chemokine.

Both a strength and a limitation of our study is that it is composed 100% of African Americans; the frequency of alleles or patterns of linkage disequilibrium differ between races with different population histories.[34,35] An and colleagues[9] have shown that European Americans have a higher frequency of the *RANTES* R1 haplotype as compared with African Americans (frequency = 0.77 and 0.57, respectively), which may make the effects of the *RANTES* haplotypes on elevated HIV-1 plasma RNA more prominent among African Americans. The association of the *RANTES* R1 haplotype with HIV-1 plasma RNA may differ between populations because of the differences in allele frequencies and haplotype structure both for the *RANTES* gene and at other known and unknown AIDS-modifying genes, however. As an example, the protective factor *CCR5* Δ32 is carried by approximately 20% of people of European origin and is virtually absent from all other geographic groups. These studies affirm the necessity of investigating the influence of genetic factors in human diseases in populations of different geographic origins.

Implications of measurable differences in HIV-1 viral load by genetic variants are important for a better understanding of HIV-1 pathogenesis and the design of therapeutics and a vaccine. Because RANTES can alter the level of HIV-1 plasma RNA in newly infected individuals, it should be considered as a potential treatment. Current studies into the use of RANTES derivatives as HIV suppressor agents should be encouraged by the apparent protective effect of *RANTES* R1 haplotypes on initial HIV-1 plasma RNA as seen here. Further cohort studies are necessary to identify the complete effects of the *RANTES* haplotypes so that targeted treatment strategies can be developed.

ACKNOWLEDGMENTS

The authors thank the participants of the ALIVE cohort.

REFERENCES

1. Frade JM, Llorente M, Mellado M, et al. The amino-terminal domain of the CCR2 chemokine receptor acts as coreceptor for HIV-1 infection. *J Clin Invest*. 1997;100:497–502.
2. Cocchi F, DeVico AL, Garzino-Demo A, et al. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science*. 1995;270:1811–1815.
3. Aukrust P, Muller F, Froland SS. Circulating levels of RANTES in human immunodeficiency virus type 1 infection: effect of potent antiretroviral therapy. *J Infect Dis*. 1998;177:1091–1096.

4. Paxton WA, Martin SR, Tse D, et al. Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposure. *Nat Med*. 1996;2:412–417.
5. Paxton WA, Liu R, Kang S, et al. Reduced HIV-1 infectability of CD4+ lymphocytes from exposed-uninfected individuals: association with low expression of CCR5 and high production of beta-chemokines. *Virology*. 1998;244:66–73.
6. Zagury D, Lachgar A, Chams V, et al. C-C chemokines, pivotal in protection against HIV type 1 infection. *Proc Natl Acad Sci USA*. 1998;95: 3857–3861.
7. Garzino-Demo A, Moss RB, Margolick JB, et al. Spontaneous and antigen-induced production of HIV-inhibitory beta-chemokines are associated with AIDS-free status. *Proc Natl Acad Sci USA*. 1999;96:11986–11991.
8. An P, Martin MP, Nelson GW, et al. Influence of CCR5 promoter haplotypes on AIDS progression in African Americans. *AIDS*. 2000;14: 2117–2122.
9. An P, Nelson GW, Wang L, et al. Modulating influence on HIV/AIDS by interacting RANTES gene variants. *Proc Natl Acad Sci USA*. 2002;99: 10002–10007.
10. Biti R, French R, Young J, et al. HIV-1 infection in an individual homozygous for the CCR5 deletion allele. *Nat Med*. 1997;3:252–253.
11. Liu H, Chao D, Nakayama EE, et al. Polymorphism in RANTES chemokine promoter affects HIV-1 disease progression. *Proc Natl Acad Sci USA*. 1999;96:4581–4585.
12. McDermott DH, Beecroft MJ, Kleeberger CA, et al. Chemokine RANTES promoter polymorphism affects risk of both HIV infection and disease progression in the Multicenter AIDS Cohort Study. *AIDS*. 2000;14:2671–2678.
13. Mummidi S, Ahuja SS, Gonzalez E, et al. Genealogy of the CCR5 locus and chemokine system gene variants associated with altered rates of HIV-1 disease progression. *Nat Med*. 1998;4:786–793.
14. Smith MW, Dean M, Carrington M, et al. Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. *Science*. 1997;277: 959–965.
15. van Rij RP, Broersen S, Goudsmit J, et al. The role of a stromal cell-derived factor-1 chemokine gene variant in the clinical course of HIV-1 infection. *AIDS*. 1998;12(Suppl):F85–F90.
16. Winkler C, Modi W, Smith MW, et al. Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. ALIVE Study, Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC). *Science*. 1998;279:389–393.
17. Daar ES, Lynn H, Donfield S, et al. Effects of plasma HIV RNA, $CD4^+$ T lymphocytes, and the chemokine receptors CCR5 and CCR2b on HIV disease progression in hemophiliacs. Hemophilia Growth and Development Study. *J Acquir Immune Defic Syndr*. 1999;21:317–325.
18. Katzenstein TL, Eugen-Olsen J, Hofmann B, et al. HIV-infected individuals with the CCR delta32/CCR5 genotype have lower HIV RNA levels and higher CD4 cell counts in the early years of the infection than do patients with the wild type. Copenhagen AIDS Cohort Study Group. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1997;16:10–14.
19. Meyer L, Magierowska M, Hubert JB, et al. Early protective effect of CCR-5 delta 32 heterozygosity on HIV-1 disease progression: relationship with viral load. The SEROCO Study Group. *AIDS*. 1997;11:F73–F78.
20. Martin MP, Dean M, Smith MW, et al. Genetic acceleration of AIDS progression by a promoter variant of CCR5. *Science*. 1998;282:1907–1911.
21. Tang J, Rivers C, Karita E, et al. Allelic variants of human beta-chemokine receptor 5 (CCR5) promoter: evolutionary relationships and predictable associations with HIV-1 disease progression. *Genes Immun*. 1999;1:20–27.
22. McDermott DH, Zimmerman PA, Guignard F, et al. CCR5 promoter polymorphism and HIV-1 disease progression. Multicenter AIDS Cohort Study (MACS). *Lancet*. 1998;352:866–870.
23. Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med*. 1997;126:946–954.
24. Vlahov D, Graham N, Hoover D, et al. Prognostic indicators for AIDS and infectious disease death in HIV-infected injection drug users: plasma viral load and CD4+ cell count. *JAMA*. 1998;279:35–40.
25. Vlahov D, Anthony JC, Munoz A, et al. The ALIVE study, a longitudinal study of HIV-1 infection in intravenous drug users: description of methods and characteristics of participants. *NIDA Res Monogr*. 1991;109:75–100.
26. Gonzalez E, Bamshad M, Sato N, et al. Race-specific HIV-1 disease-modifying effects associated with CCR5 haplotypes. *Proc Natl Acad Sci USA*. 1999;96:12004–12009.
27. Dean M, Carrington M, Winkler C, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science*. 1996;273:1856–1862.
28. Farzadegan H, Hoover DR, Astemborski J, et al. Sex differences in HIV-1 viral load and progression to AIDS. *Lancet*. 1998;352:1510–1514.
29. Sterling TR, Lyles CM, Vlahov D, et al. Sex differences in longitudinal human immunodeficiency virus type 1 RNA levels among seroconverters. *J Infect Dis*. 1999;180:666–672.
30. Sterling TR, Vlahov D, Astemborski J, et al. Initial plasma HIV-1 RNA levels and progression to AIDS in women and men. *N Engl J Med*. 2001; 344:720–725.
31. Gonzalez E, Dhanda R, Bamshad M, et al. Global survey of genetic variation in CCR5, RANTES, and MIP-1alpha: impact on the epidemiology of the HIV-1 pandemic. *Proc Natl Acad Sci USA*. 2001;98:5199–5204.
32. Tang J, Shelton B, Makhatadze NJ, et al. Distribution of chemokine receptor CCR2 and CCR5 genotypes and their relative contribution to human immunodeficiency virus type 1 (HIV-1) seroconversion, early HIV-1 RNA concentration in plasma, and later disease progression. *J Virol*. 2002;76:662–672.
33. Tang J, Wilson CM, Schaen M, et al. CCR2 and CCR5 genotypes in HIV type 1-infected adolescents: limited contributions to variability in plasma HIV type 1 RNA concentration in the absence of antiretroviral therapy. *AIDS Res Hum Retroviruses*. 2002;18:403–412.
34. Cavalli-Sforza LL, Feldman MW. The application of molecular genetic approaches to the study of human evolution. *Nat Genet*. 2003;33(Suppl): 266–275.
35. Goldstein DB, Chikhi L. Human migrations and population structure: what we know and why it matters. *Annu Rev Genomics Hum Genet*. 2002; 3:129–152.

